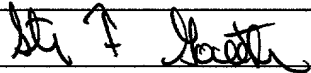


08/901,225

EXPRESS MAIL CERTIFICATE			
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.			
Typed or Printed Name	Steven F. Goldstein		EL 923 480 185 US
Signature			Date June 5, 2001

<b>PRELIMINARY AMENDMENT</b>  Address to: Box Patent Application Assistant Commissioner for Patents Washington, D.C. 20231	Attorney Docket Confirmation No.	IRVN001DIV
	First Named Inventor	Hiserodt, et al.
	Application Number	Unassigned
	Filing Date	Herewith
	Group Art Unit	Unassigned
	Examiner Name	Unassigned
	Title	"Cancer Immunotherapy Using Autologous Tumor Cells Combined with Allogeneic Cytokine-Secreting Cells"

Sir:

Prior to examination of the application on the merits, please enter the following amendments:

### AMENDMENTS

#### IN THE TITLE:

Replace the title with the following new title:

CANCER IMMUNOTHERAPY USING AUTOLOGOUS TUMOR CELLS COMBINED WITH CELLS  
EXPRESSING A MEMBRANE CYTOKINE

#### IN THE SPECIFICATION:

Replace the paragraph beginning at page 1, line 8 with the following rewritten paragraph:

This application is a divisional of U.S. Application Serial No. 08/901,225, filed July 24, 1997, now pending, which application claims the priority benefit of provisional U.S. Application Serial Nos. 60/023,108, filed July 25, 1996, now abandoned; and 60/029,286, filed October 29, 1996, now abandoned. The afore-listed applications are hereby incorporated herein by reference in their entirety.

Please replace the paragraph beginning at page 12, line 19, with the following rewritten paragraph:

--**Figures 2A-C** are graphs showing the effects of irradiation on the IL-4 secreting tumor cell line UC1 107E IL-4 GS. Figure 2A shows the growth pattern of cells given 5,000 (□) or 10,000 (■) rads. Figures 2B & 2C show IL-4 detected by ELISA in the culture medium expressed as total concentration (Figure 2B) or per cell (Figure 2C) Various times after irradiation.—

Please replace the paragraph beginning at page 12, line 24, with the following rewritten paragraph:

--**Figure 3A-C** are a series of FACS analysis profiles (incidence versus fluorescence intensity) revealing expression of various surface antigen by UCI 107E IL-4 GS, before or after irradiation with 5,000 or 10,000 rads.—

Please replace the paragraph beginning at page 47, line 23, with the following rewritten paragraph:

--Results of this experiment are shown in Figures 2A-C. Cells irradiated with between 2,500 and 10,000 rads remained viable for about 8 days but all the cells were dead by 3 weeks. Cells irradiated with 1,000 rads recuperated and continued to proliferate. Levels of cytokine production were detectable for 8 days at all doses and closely paralleled the number of viable cells. Figure 2B shows IL-4 production after irradiation at 5,000 rads (□) or 10,000 rads (■) in three separate experiments. Figure 2C shows IL-4 production standardized in pg/ml/10<sup>5</sup> cells/48 hr by UCI 107E IL-4 GS cells after irradiation at 5,000 or 10,000 rads in two separate experiments. No statistically significant differences in survival were seen among cells irradiated with 2,500, 5,000, and 10,000 rads on days 2 (p = 0.72), 4 (p = 0.14), 6 (p = 0.10), and 8 (p = 0.3).—

Please replace the paragraph beginning at page 48, line 15, with the following rewritten paragraph:

--The expression of surface antigens detected by FACS analysis is illustrated in Figures 3A-C. Parental cells, vector controls, and 107E IL-4 GS cells constitutively express MHC class I

antigens and Her-2/neu, but did not express MHC class II antigens, CA-125, ICAM-1, or IL-4 receptors. Expression of surface antigens was also determined at 2 or 8 days after irradiation. MHC class I antigen and Her-2/new antigen expression increased significantly at all radiation doses, and tended towards higher expression at higher doses. Irradiation did not induce expression of HLA class II antigens, ICAM-I, or CA-125.--

**IN THE CLAIMS**

Cancel original claims 1-30 without prejudice. Add new claims 31-62.

31. A composition comprising a cell genetically altered to express a cytokine stably associated in the cell outer membrane, or the progeny of such a cell, which upon administration to a subject is effective in treating a neoplastic disease or eliciting an anti-tumor immunological response in the subject.
32. The composition of claim 31, wherein the cytokine is selected from the group consisting of IL-4, GM-CSF, IL-2, TNF- $\alpha$ , and M-CSF.
33. The composition of claim 31, wherein the cell is a cancer cell.
34. The composition of claim 31, wherein the cell is from a cancer of the same tissue type as a tumor in the subject.
35. The composition of claim 33, wherein the cancer is an ovarian cancer or a brain cancer.
36. The composition of claim 31, wherein the cell is allogeneic to the subject.
37. The composition of claim 31, wherein the cell is histocompatibly identical to the subject.

38. The composition of claim 31, further comprising a tumor-associated antigen, wherein the combination of the cytokine and the tumor-associated antigen in the composition is effective in treating a neoplastic disease or eliciting an anti-tumor immunological response in the subject.
39. The composition of claim 38, wherein the tumor-associated antigen is obtained from a cell autologous to the subject.
40. The composition of claim 38, wherein the tumor-associated antigen is expressed by the same cells expressing the membrane-associated cytokine.
41. The composition of claim 38, comprising a combination of:
- a) the cell expressing the membrane-associated cytokine; and
  - b) a tumor cell autologous to the subject;
- wherein the combination is effective in treating a neoplastic disease or eliciting an anti-tumor immunological response in the subject.
42. The composition of claim 41, wherein the tumor cell is a primary tumor cell dispersed from a solid tumor obtained from the subject.
43. The composition of claim 41, wherein the tumor cell is a glioma, a glioblastoma, a gliosarcoma, an astrocytoma, or an ovarian cancer cell.
44. The composition of claim 41, wherein the tumor cell is inactivated.
45. The composition of claim 41, wherein the cell expressing the membrane-associated cytokine is inactivated.
46. The composition of claim 41, wherein the cell produces a secreted cytokine in addition to the cytokine stably associated in the outer membrane.

47. The composition of claim 41, wherein a majority of the cytokine produced by the cell is present on the outer membrane of the cell.
48. The composition of claim 41, wherein the cytokine is selected from the group consisting of IL-4, GM-CSF, IL-2, TNF- $\alpha$ , and M-CSF.
49. A composition comprising a tumor associated antigen and a population of cells expressing a transmembrane cytokine at a level sufficient to stimulate an immune response to the tumor associated antigen.
50. A unit dose of the composition according to claim 31, wherein the number of cells is at least about  $5 \times 10^6$  but not more than about  $2 \times 10^8$ .
51. The composition of claim 31, wherein the cell is a human cell.
52. The composition of claim 31, wherein the cytokine naturally occurs as a membrane cytokine.
53. The composition of claim 31, wherein the cytokine is a fusion protein comprising a heterologous transmembrane region.
54. The composition of claim 31, wherein the cell has been transduced with a retroviral expression vector, or is the progeny of such a cell.
55. A method for producing the composition of claim 31, comprising transducing the cell with an expression vector encoding the membrane-associated cytokine.
56. The method of claim 55, wherein the expression vector is a retroviral vector.
57. The method of claim 55, wherein the cytokine is selected from the group consisting of IL-4, GM-CSF, IL-2, TNF- $\alpha$ , and M-CSF.

- 6

**REMARKS UNDER 37 CFR § 1.111**

**Formal Matters**

Claims 31-62 are pending after entry of the amendments set forth herein.

Claims 1-30 are canceled without prejudice to renewal, without acquiescing to any rejection that may have been applied to the claims, and without intent to abandon any subject matter encompassed in the claims.

The amendments to the specification are made solely to make the figure numbering match the figure numbering in the formal drawings submitted herewith. Applicants respectfully request entry of the amendments.

Support for new claims 31-62 is found throughout the specification, and particularly at, for example, page 11, line 15 to page 12, line 6; page 21, lines 16-19; page 24, lines 1-4 and lines 17-23; page 26, lines 24-25; page 27, lines 12-20; page 29, lines 3-14; page 29, line 24 to page 30, line 3; page 30, lines 19-20; page 34, lines 24-27; page 35, lines 21-27; page 37, line 21; page 37, line 26 to page 38, line 2; page 39, lines 16-17; and in the Examples (page 43, line 14 to page 71, line 7).

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Applicants respectfully request reconsideration of the application in view of the amendments and remarks made herein.

No new matter has been added.

**Conclusion**

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number IRVN001DIV.

Respectfully submitted,  
BOZICEVIC, FIELD & FRANCIS LLP

Date:

June 5, 2001

By:

Carol L. Francis  
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Registration No. 36,513

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE TITLE:**

CANCER IMMUNOTHERAPY USING AUTOLOGOUS TUMOR CELLS COMBINED WITH [ALLOGENEIC  
CYTOKINE-SECRETING CELLS] CELLS EXPRESSING A MEMBRANE CYTOKINE

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